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Original Research Article

Yeast Enriched with Selenium: A Promising Source of Selenomethionine and Seleno-Proteins

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HIGHLIGHTS

- Selenomethionine is an important amino acid that has a significant role against oxidative stress.
- Addition of inorganic selenium to the yeast media culture leads to production of the selenomethionine.
- Saccharomyces cerevisiae is one of the best organisms for selenium biotransformation.

Keywords:
Biotransformation
Selenium
Selenomethionine
Seleno-protein
Yeast

ABSTRACT

Organic selenium compound such as selenomethionine plays a significant function in response to oxidative stress. Currently *Saccharomyces cerevisiae* is one of the best organisms that has ability to accumulate selenomethionine and selenium biotransformation. Addition of mineral selenium to medium culture is a very common practice in order to produce the selenomethionine and Seleno-proteins. Due to the toxicity of selenium for yeasts, selenium tolerant yeast isolation procedures are required. The aim of this investigation was to separate indigenous selenium tolerant *S.cerevisiae* strains which will not be affected by high selenium concentrations and are able to produce high levels of selenomethionine. In this study, 85 samples were collected from fermentative fruit. Screening was carried out in order to select high yeast cell density and also high selenomethionine accumulation. After confirming yeast strains, selected strains were cultured at a concentration of 25 mg/L sodium selenite and selenomethionine content was measured after 48 hours. The S18 isolate showed had maximum biomass production and selenomethionine accumulation (2655 ppm) and (3.73 g/L) compared to the other isolates.

Introduction

Selenium (Se) has been shown to be a vital micronutrient of human diets (Bierla et al., 2012; Kieliszek and Blazejak, 2013). This element has many beneficial health effects. This element was discovered by Doctor Jons Jakob Berzelius in 1817 (Rajashree and Muthukumar, 2013a; Kokarnig et al., 2015). Selenium incorporates in 25 essential selenoproteins and several enzymes (Kieliszek and Blazejak, 2013; Oraby, et al. 2015). Selenium was known as an essential part of glutathione peroxidase (Marinescu et al., 2011; Rajashree and Muthukumar, 2013b). This enzyme in company with superoxide dismutase and catalase (antioxidant enzymes) has important antioxidant and detoxification functions and protects cells from the oxidation damage of the free radicals (Rajashree and Muthukumar, 2013b; Rajashree

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and Muthukumar, 2013a).Selenium deficiencies are related to the several diseases including cancer, heart disease, infertility, and reversible cardiomyopathy identified as Keshan disease (Rajashree and Muthukumar, 2013a; Yang, 2013; Zare et al., 2014). Therefore, consumption of adequate organic selenium is essential for human health (Oraby et al., 2015). Selenomethionine is the form of organic selenium in which it enters the food chain of humans. Since humans cannot produce selenomethionine, this amino acid fulfills an essential amino acid (Alimadadi et al., 2016). Lots of people in many countries have selenium deficiency and this subject has been making the increasing attention in the selenium supplementation of food (Bierla et al., 2012). Under Inappropriate conditions, yeast cells (*Saccharomyces cerevisiae*) are capable to convert inorganic selenium into organic selenium ingredients (selenomethionine). This biotransformation was done by some enzymes activated in the presence of selenium in culture (Yin et al., 2009; Sanchez et al., 2012; Rajashree and Muthukumar, 2013b). So, creation of selenium enriched yeast is the best choice to provide the selenomethionine demands of human requirements (Bierla et al., 2012).

In this study, we have screened the selenium-tolerant *Saccharomyces cerevisiae* from 100 samples of Iranian indigenous yeast. The main goal of this investigation was to select yeast isolates with high Seleno-protein content that have high selenomethionine amino acid.

Materials and Methods

Materials

Master Mix PCR was obtained from Genaxxon Co. (Germany). SD broth, Sabouraud dextrose (SD) agar, yeast-extract glucose chloramphenicol (YGC) agar and sodium selenite were supplied by Merck Co. (Germany). Other liquid and powder chemicals were sold from Merck Co. (Germany). The standard yeast *Saccharomyces cerevisiae* (PTCC 5157) was obtained from Iranian Research Organization for Science and Technology (IROST), Iran and was used as standard strain.

Yeast isolation and identification

85 samples were collected from fermentable fruits (grape, apple and dates). One gram of each fruits was added into 100 ml flask containing 50 ml of normal saline buffer. After serial dilution, yeast strains were isolated with streaking plate method on YGC agar incubated at 30°C for 3-5 days (Tahmasebi et al., 2016). The yeast strain was maintained on SD broth with %20 glycerol at -80 ºC. The yeast colonies were identified according to the criteria of Kurtzman and Fell. Yeasts were identified by biochemical, physiological, and morphological properties, such as the ability to hydrolyze urea as well as ferment sugars (Glucose, Sucrose, Maltose, Lactose, and Galactose) (Hampsey, 1997; Kurtzman et al., 2011). In addition, molecular identification was done with PCR using specific primers: SC1 (5'-AACGGTGAGAGATTTCTGTGC-3') and SC2 (5'-AGCTGGCAGTATTCCCACAG-3') (Josepa et al., 2000). Finally, two isolates with highest tolerance against selenium were characterized with sequencing of ITS region (including 5.8S rRNA gene) and the LSU rRNA gene D1/D2 domain of 26S rDNA (Alimadadi et al., 2016). Following, these sequences were compared with those included in the GenBank database by the Basic Local Alignment Search Tool (BLAST).

Screening for yeast with high solenoid protein and high selenomethionine

This step was done to select the yeast strains with maximum solenoid protein content and high selenomethionine amino acid. For this goal, the yeast strains confirmed in previous step were grown in SD broth containing 25 mg/l of sodium selenite (Na2SeO3), and incubated at 30°C for 24 hours. Next, the cultures were centrifuged at 8000 g for 10 minutes and washed three times by deionized water for the removal of selenium adsorbed on the cell surface. The cells were dried at 85 °C to achieve a constant weight (2 hours) and reweighed. Determination of selenomethionine content was done according to the atomic absorption spectroscopy (AAS) method. 0.2 g of the dried yeast was digested with 10 ml of concentrated nitric acid (65%) for 15 min at 105°C in a digestive flask. After that, the solution was cooled and for completing the digestion process, the solution was heated with 2 ml of concentrated hydrochloric acid for 10 min at 80°C. This solution was used for total selenium determination by AAS. To measure the inorganic selenium, 0.2 g of the dried yeast in ultra-pure water was extracted in boiling bath for 1 h. After that, the mixture was centrifuged at 8000 g for 15 min and the supernatant was filtrated and used for inorganic selenium determination by AAS (Esmaeili et al., 2012). Standard curve with known amounts of selenium were generated to determine total and inorganic selenium. Calculation of selenomethionine yield was achieved from the difference between the total and inorganic Se yield (Esmaeili et al., 2012).

Results

Isolation and characterization

Among 85 samples, 40 isolates were selected after isolation and characterization. Yeast isolates were named S1 to S40. All isolates were identified by the biochemical and physiological tests (The criteria of Kurtzman and Fell) (Hampsey, 1997, Kurtzman et al., 2011). Next, identification of the isolates was done by molecular tests with PCR with specific primers and sequencing of ITS

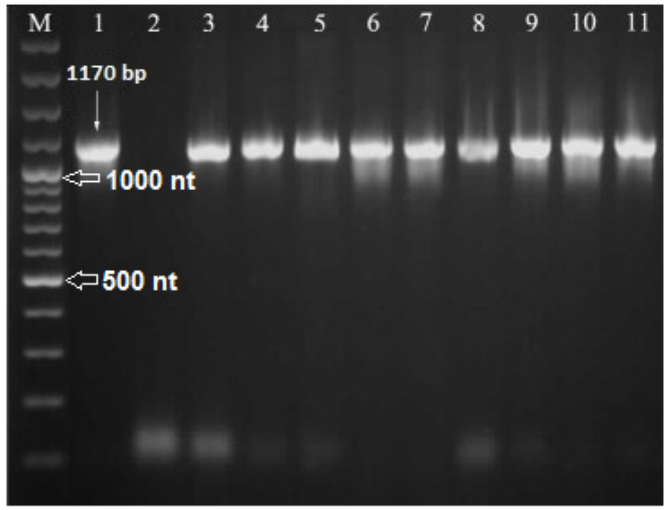

Figure 1. The PCR product of reference S. cerevisiae strain and S. cerevisiae isolates. M: 100 bp plus marker, line 1: reference strain S. cerevisiae, line 2: negative control, line 3 to 12: respectively S5, S12, S18, S19, S24, S31, S34, S36, S38, and S40 isolates.

region (including 5.8S rRNA gene) and the LSU rRNA gene D1/D2 domain of 26S rDNA. The results showed that PCR products were about 1170 bp and there was no considerable difference compared with the reference strain (Fig. 1). Two isolates (S5 and S18) with the best selenomethionine and biomass creation were chosen for further characterization. The results of the sequencing LSU (Large Subunit), rRNA gene D1/D2 domains, and ITS (Internal transcribed spacer) region revealed that S5 and S18 isolates must be strains of *S. cerevisiae* (data not shown).

Screening for yeast with high solenoid protein and high selenomethionine

The confirmed yeast isolates were grown in SD broth containing 25 mg/l of sodium selenite (Na2SeO3), incubated at 30°C for 24 hours and then analyzed for the selenomethionine content. Selenomethionine accumulation of the S5, S12, S18, S19, S24, and S38 isolates were 2600, 1579, 2655, 1170, 1630, and 1470ppm respectively. The S18 isolate showed maximum selenomethionine content (high selenium biotransformation) (2655 ppm) and biomass production (3.73 g/L) compared to the other isolates (Fig. 2).

Discussion

As a result, finding yeast strains resistant to selenium can be very useful. The yeast isolates were exposed to mineral selenium at 25 mg/L concentrations. While exposed to the selection pressure, yeast cells were capable to obtain new metabolic capacities. So, yeast cells can elevate resistance to toxic metals such as selenium (Yang et al., 2004). Only 5 yeast cells were gained to acceptable biomass (above 3 g/L dry cell weight) and among them only S5 and S18 have good selenomethionine content (above 2500 ppm organic selenium). Even though the yeast isolates can grow and accumulate biomass in the presence of selenium, their ability to selenium bio transformation is important.

The five yeast isolates from the prior step were subjected to the determination of selenomethionine content. S5 and S18 isolates has given high selenomethionine content and biomass production. While S24 isolate had appropriate production of biomass, it had low selenomethionine content (selenium biotransformation). Also, the S12 isolate had high biomass production and low selenium biotransformation. Other studies had shown that the amount of selenomethionine yeast cells was between 500 to 3000 ppm (Rajashree and Muthukumar, 2013a). Greatest biotransformation of selenium (2718 ppm) was obtained with synthetic medium by Muthukumar and Rajashree (2013). Antoneta and Marinescu (2011) reported selenomethionine production in the range of 300 to 2200 ppm in yeast cells.

This is achieved by supplementation of the medium with 30 to 180µg/ ml sodium selenite. Also Suhajda et al. (2000) were able to produce yeast with 1200 to 1400 µg selenomethionine per gram dried yeast. Finally, in this investigation, the results demonstrated that strain (S18) is capable of producing 2655 ppm selenomethionine (organic selenium) in presence of 25 mg/L of selenium, which is higher than the other isolates. This increase in selenomethionine content was established just with screening and without manipulating the media.

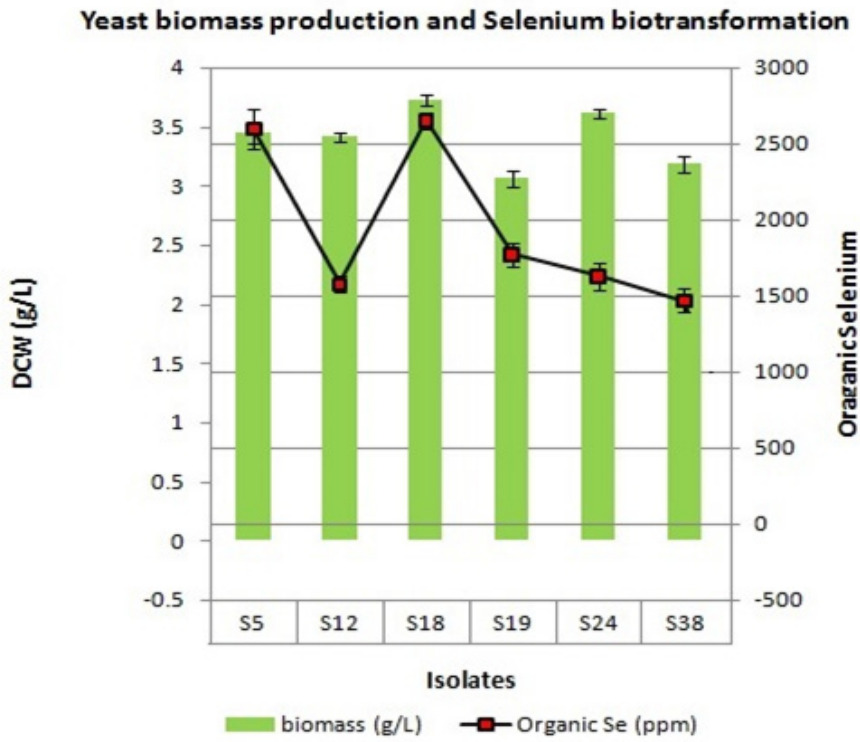

Figure 2. Screening of selenium tolerant yeast isolate for high yeast biomass and organic selenium production.

Conclusion

Although selenium species are produced by *Saccharomyces* and *Lactobacillus*, selenomethionine, which is the main selenium-containing amino acid, is created in *S. cerevisiae* (Yang et al., 2004). *S. cerevisiae* is also known as one of the best probiotics in human diet (Josepa et al., 2000). It is attractive that organic selenium such as selenomethionine and solenoid protein has a very low toxicity and high bioavailability compared mineral selenium (Rajashree and Muthukumar, 2013a). In this study, we isolated an indigenous *S. cerevisiae* with high selenomethionine and Seleno-protein content. This Seleno-yeast can be helpful for cancer prevention.

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Competing Interests

The authors declare that there are no conflicts of interest.

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